600-1-280N

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Simon S. et al. EXAMINER: Wehbe, Anne Marie Sabrina

SERIAL NO.: 09/982,120 ART UNIT: 1632

Filed: October 17, 2001

For: ANIMALS, CELLS AND METHODS FOR PRODUCTION OF DETECTABLY-LABELED ANTIBODIES

DECLARATION UNDER 37 C.F.R. 1.132

COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VIRGINIA 22313-1450

SIR:

I, SANFORD SIMON, hereby declare and state that:

1. I am a Professor in the Department of Cellular Biophysics at Rockefeller University having received my Ph.D. degree from New York University Medical Center in 1984. After that I was a postdoctoral fellow at Rockefeller University. My full curriculum vitae is attached hereto as EXHIBIT A.

2. My principal area of research is Cell Biology, and among other positions I serve as reviewer in numerous funding agencies of many countries, including the United States (NSF, NIH), Germany, England, Israel and Canada. I also have served as reviewer for numerous scientific journals including Nature, Cell, Science, Journal of Cell Biology, Nature Medicine, Nature Biotechnology, Nature Cell Biology, PLOS and many others.

3. In the course of my activities, I have been listed as inventor on several patent applications, including the one noted above entitled "ANIMALS, CELLS AND METHODS FOR PRODUCTION OF DETECTABLY-LABELED ANTIBODIES",

having U.S. Serial Number 09/982,120, which claims priority to U.S. provisional application Serial Number 60/241,053, filed on October 17, 2000.

4. I have reviewed the present application, including subject matter that may be directly applied to genetically engineered animals and antibody producing cells obtained from these animals, and generating detectably labeled antibodies specific for preselected antigens.

5. Furthermore, based on what was known prior to October 17, 2000, as demonstrated by Fell et al. (U.S. patent No. 5,202,238), Casey et al. (June 2000, Protein Engineering Vol. 13 (6):445-452) and Rajewsky et al. (U.S. patent No. 6,570,061), a Ph.D. scientist in the field of Immunology would not have believed that one could produce the animals and cells disclosed in the claims of the pending application.

6. Subsequent to the filing of this patent application, I consulted with several immunologists to determine the best way of testing whether the immune system of the mice we made was fully functional. These scientists were not aware of the fact that we had already:

- Demonstrated that Ab-GFP fusion proteins could be made
- Demonstrated that Ab-GFP fusion proteins have the same binding kinetics of native antibody
- Demonstrated that we could make Ab-GFP fusion proteins in mice
- Demonstrated that we could replace one of the exons of the native mouse Kappa chain with an exon that has a GFP such that all Kappa light chains have a GFP.

The scientists I consulted provided two reasons for why they believed my experimental approach would fail. These reasons are summarized in paragraphs 7 and 8.

GFP disrupts protein function.

7. There are many examples of Green Fluorescent Protein (GFP) fusions in which the resulting fusion protein is either:

- not made properly (it is degraded);

- made, but does not fold properly;

- made, but undergoes aggregation;

- made, but the GFP interferes with a sorting signal (GFPs are rarely put into the middle of a protein – the protein does not fold. Instead they are usually put at the amino or carboxyl terminus which are the "free" ends. However, since they are the free ends, they are also the positions which have sorting signals. For example, almost every membrane protein (and antibodies go through phases of being membrane proteins) uses its carboxyl terminus as a sorting signal.);

- made and sorted, but has altered kinetics which disrupts cell function (for example, CFTR has improper kinetics with any kind of tag at its amino or carboxyl terminus). In numerous cases a GFP fusion has a "dominant negative" effect: It completely blocks even the activity of the wild-type non-fusion protein (Han W, Rhee JS, Maximov A, Lin W, Hammer RE, Rosenmund C, Sudhof TC., C-terminal ECFP fusion impairs synaptotagmin 1 function: crowding out synaptotagmin, J Biol Chem. (2005) Feb 11; 280(6):5089-100 (EXHIBIT B))

- There is the additional problem that when GFPs are expressed in an animal on the outside of a cell (not inside the cytosol), an immune response is triggered.

The Ab genome goes through numerous recombinations in development of the immune system and the antibody response.

8. It is known that even the slightest aberrations in aspect of the process (such as timing or switching of Ab classes) result in the B-cell undergoing apoptosis. Given what was known about the extensive problems with GFP fusion, it would not have been possible to produce a viable mouse or a mouse with an intact immune response.

Others have failed when trying to make similar inventions for several reasons

9. Other scientists have attempted to produce fusions similar to those described in our present patent application and have failed for various reasons. The following is an annotated list of some of the problems that have occurred when a protein has been expressed as a fusion to GFP:

Dominant Negative Effects of GFP Fusions

10. The term Dominant Negative refers to a situation whereby for example, the GFP-fusion protein may actually over-ride and block the function of the native protein. Examples of this are reported as follows.

(1) GFP fused to the amino terminus of caveolin1 is a dominant negative inhibitor of viral entry (e.g. SV40):

- Daecke J, Fackler OT, Dittmar MT, Krausslich HG., (2005), Involvement of clathrin-mediated endocytosis in human immunodeficiency virus type 1 entry. J Virol. Feb;79(3):1581-94.

- Pelkmans, L., Kartenbeck, J., and Helenius, A., (2001), Caveolar endocytosis of simian virus 40 reveals a new two-step vesicular-transport pathway to the ER. Nat. Cell Biol. 3, 473- 483 (EXHIBIT C).

(2) GFP fused to the carboxyl terminus of synaptotagmin (which we actively study) blocks the function of synapses:

-Han W, Rhee JS, Maximov A, Lin W, Hammer RE, Rosenmund C, Sudhof TC, (2005), C-terminal ECFP fusion impairs synaptotagmin 1 function: crowding out synaptotagmin 1, J Biol Chem. Feb 11; 280(6): 5089-100, (EXHIBIT B)

(3) Yeast cells grow more slowly when a component of the spindle pole body, NUF1, is expressed as a fusion to GFP:

-Observation by T.N. David published in: A. B. Cubitt, R. Heim, S. R. Adams, A. E. Boyd, L. A. Gross, and R. Y. Tsien, (1995), Understanding, improving and using green fluorescent proteins. Trends Biochem.Sci. 20 (11):448-455 (EXHIBIT D).

GFP Fusions may not be functional

11. Many people have tried to make a GFP fusion to the protein Sar1. None of them have worked (personal communication). My laboratory has made GFP fusions to BCRP (a drug resistance protein) and we have found that GFP fusions to either the amino or carboxyl terminus are not functional. Expression of synchollin inhibits secretion, but a GFP fusion to synchollin is without effect. The authors of this work conclude that GFP put anywhere on the protein affects its function. (B. Wasle, L. B. Hays, C. J. Rhodes, and J. M. Edwardson, (2004), Syncollin inhibits regulated corticotropin secretion from AtT-20 cells through a reduction in the secretory vesicle population, Biochem.J. 380 (Pt 3):897-905, (EXHIBIT E)). Furthermore, actin fused to GFP does not function. (T. Doyle and D. Botstein, (1996), Movement of yeast cortical actin cytoskeleton visualized in vivo, Proc.Natl.Acad.Sci.U.S.A 93 (9):3886-3891, (EXHIBIT F))

GFP fusions may alter function

12. Any tag (from a small myc tag to a GFP) put on the amino terminus of CFTR (the cystic fibrosis transmembrane regulator) disrupts the function of the protein. (K. W. Chan, L. Csanady, D. Seto-Young, A. C. Nairn, and D. C. Gadsby. Severed molecules functionally define the boundaries of the cystic fibrosis transmembrane conductance regulator's NH(2)-terminal nucleotide binding domain, (2000), J.Gen.Physiol, 116 (2):163-180). Any tag on the carboxyl terminus disrupts sorting (D. Gadsby, personal communication).

GFP tags induce an immune response from the animal

13. See abstract of S. German-Retana, T. Candresse, E. Alias, R. P. Delbos, and O. Le Gall. Effects of green fluorescent protein or beta-glucuronidase tagging on the accumulation and pathogenicity of a resistance-breaking Lettuce mosaic virus isolate in susceptible and resistant lettuce cultivars. (Mol. Plant Microbe Interact. 13 (3):316-324, 2000 (EXHIBIT G)).

GFP tags may induce aggregation

14. GFP normally is a dimer, and dsRed is normally a tetramer. GFPs tags often induce proteins to dimerize thereby affecting their function. Given the vast collected experience demonstrating that GFP fusion proteins usually do not function, in my opinion it would involve considerable planning and testing to design a GFP fusion that could potentially be synthesized without resulting in aggregation and degradation; properly folded and assembled with the other subunits of the antibody; properly targeted through the secretory pathway and functional at the surface. Furthermore, it would be difficult to achieve success of all of the above without disrupting the rearrangement reactions that occur during the generation of B-cells and the immune reaction. Thus, the numerous attempts at producing animals or cells similar to the fusions we have made, and the failures associated with these attempts, would provide doubt that the information in the references of Fell et al., Casey et al. and Rajewsky et al. could serve to instruct someone to produce the cellular constructs or the transgenic animals as we have achieved and shown in our present application.

15. I have considerable experience in the following techniques:

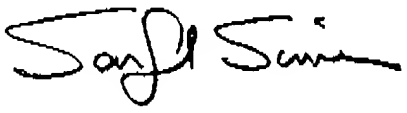
a) The sorting and targeting of proteins in the secretory system (some of my work on this subject was cited in the 1999 Nobel Prize for Physiology or Medicine and highlighted in the Nobel Lecture by Gunter Blobel): (Simon and Blobel, 1991;Simon and Blobel, 1992;Borel and Simon, 1996;Schmoranzer et al., 2000;De Souza and Simon, 2002;Jaiswal et al., 2002;Kanner et al., 2002b;Kanner et al., 2002a;Rajagopal et al., 2002;Kanner et al., 2003;Kreitzer et al., 2003;Rajagopal and Simon, 2003;Schmoranzer and Simon, 2003;Schmoranzer et al., 2003;Jaiswal et al., 2004); and

b) Assaying whether GFP fusion proteins are functional: (Chen and Simon, 2000;Chen et al., 2001;Rajagopal et al., 2002;Rajagopal and Simon, 2002;Kreitzer et al., 2003;Rajagopal and Simon, 2003;Rappoport et al., 2003b;Rappoport et al., 2003a;Rappoport and Simon, 2003;Schmoranzer and Simon, 2003;Rappoport et al., 2004).

16. The technology described in the above-noted application provides many advantages. Such a genetic construct provides for the ability of this type of chimeric antibody to be useful for not only detection of the specific antigen in vitro in a diagnostic setting, but also is useful for antibody purification due to the presence of the chitin binding domain or for introduction of a toxin or radiolabel or other probe onto the antibody due to the presence of the intein.

17. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18 of the U.S. Code, Section 1001, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Dated: March 22, 2005



Sanford Simon